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DNA SEQUENCING AND CHARACTERIZATION

Murtaza M, Dawson S-J, Tsui D W Y, Gale D, Forshew T, Piskorz A M, Parkinson C, Chin S-F, Kingsbury Z, Wong A S C, Marass F, Humphray S, Hadfield J, Bentley D, Chin T M, Brenton J D, Caldas C, Rosenfeld N. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* 497;2013:108–112.

This paper demonstrates that patients with a high tumor burden may have sufficient tumor DNA in their plasma for deep sequencing to track genomic changes occurring in their tumor(s) in response to treatment. Six patients with breast, ovarian, or lung cancer are followed during treatment with the requisite drugs in this work, and changes in tumor allele fraction that are linked to the emergence of resistance to therapy are detected by exon sequencing. The procedure is minimally invasive to patients and is hoped to enable early detection of adverse treatment response by serial analysis of cancer genomes.

SMALL MOLECULE ANALYSIS AND METABOLOMICS

Gaikwad N W. Ultra performance liquid chromatography-tandem mass spectrometry method for profiling of steroid metabolome in human tissue. *Analytical Chemistry* 85;2013:4951–4960.

Methodology is described for simultaneous measurement of all classes of endogenous steroids in human tissues, plus selected exogenous steroids. Accomplishment of such measurements is made challenging by the low levels of these compounds, the diversity of their physical and chemical properties, and the large range of concentrations at which they are present. To meet these challenges, a liquid extraction procedure is adopted in place of a solid-phase protocol. The underivatized samples are then analyzed by ultra-high-performance liquid chromatography-tandem mass spectrometry, using a run time of just 12 min. The methodology is applied to human breast-tissue biopsies. Androgens, corticosteroids, progestogens, estrogens, estrogen metabolites, estrogen conjugates, and estrogen-DNA adducts are measured, along with exogenous steroid derivatives. The method is of interest to investigators wishing to perform high-throughput, parallel testing of steroids in tissues.

CARBOHYDRATES AND GLYCOCONJUGATES

Everest-Dass A, Abrahams J, Kolarich D, Packer N, Campbell M. Structural feature ions for distinguishing N- and O-linked glycan isomers by LC-ESI-IT MS/MS. *Journal of the American Society for Mass Spectrometry* 24;2013: 895–906.

Serang O, Froehlich J W, Muntel J, McDowell G, Steen H, Lee R S, Steen J A. SweetSEQer, simple de novo filtering and annotation of glycoconjugate mass spectra. *Molecular & Cellular Proteomics* 2013:1735–1740.

Strum J S, Nwosu C C, Hua S, Kronewitter S R, Seipert R R, Bachelor R J, An H J, Lebrilla C B. Automated assignments of N- and O-site specific glycosylation with extensive glycan heterogeneity of glycoprotein mixtures. *Analytical Chemistry* 85;2013:5666–5675.

Zhu Z, Hua D, Clark D F, Go E P, Desaire H. GlycoPep Detector: a tool for assigning mass spectrometry data of N-linked glycopeptides on the basis of their electron transfer dissociation spectra. *Analytical Chemistry* 85; 2013:5023–5032.

Mass spectral analysis of glycoconjugates remains a labor-intensive and challenging task for which development of automated software tools has lagged behind software for analysis of unglycosylated peptides. A number of software tools have now become available for automated

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interpretation of glycoconjugate product ion mass spectra. Everest-Dass et al. assemble a library of annotated collision-induced dissociation (CID) mass spectra from diverse glycan structures, together with diagnostic fragment ions for particular structural features. Serang et al. describe a simple tool to distinguish glycosylated from nonglycosylated peptides in digests. Strum et al. provide software to identify N- and O-linked glycosylation sites on proteins based on CID spectra. Finally, Zhu et al. present software for analysis of glycopeptides by electron transfer dissociation, a technique that cleaves the peptide portion but not the glycan portion of glycopeptides.

MASS SPECTROMETRY

Stejskal K, Potěšil D, Zdráhal Z. Suppression of peptide sample losses in autosampler vials. *Journal of Proteome Research* 12;2013:3057–3062.

Very high-sensitivity analysis of biomolecules by liquid chromotography-mass spectrometry entails chromatography of samples containing very small amounts of analyte (often single fmole) in very dilute solution (0.1 nM or less). Samples are generally loaded onto the chromatography column via an automated injector, in which they may sit for several hours awaiting analysis. Adsorptive losses on the walls of autosampler vials may be severe under such circumstances. Stejskal et al. test various combinations of autosampler vial surface and sample injection solution to minimize such losses. The combination of a polypropylene vial and a solution containing 0.0001% polyethylene glycol (PEG) or a mixture of 6 M urea and 1 M thiourea, both acidified by 0.1% formic acid, gives the best results in terms of number of peptides identified. For protein digests, these solutions afford protection from adsorptive losses over a 48-h period. Analysis of samples containing such levels of PEG over several months is not accompanied by degradation of chromatographic performance.

PROTEINS—PURIFICATION AND CHARACTERIZATION

Bai X-c, Fernandez I S, McMullan G, Scheres S H. Ribosome structures to near-atomic resolution from thirty thousand cryo-EM particles. *eLife* 2;2013:e00461.

Li X, Mooney P, Zheng S, Booth C R, Braunfeld M B, Gubbens S, Agard D A, Cheng Y. Electron counting and beam-induced motion correction enable near-atomic-resolution single-particle cryo-EM. *Nature Methods* 10; 2013:584–590.

Single-particle cryo-electron microscopy (cryo-EM) has long been used to study the three-dimensional (3D) structure of macromolecules. The technique involves immobilizing macromolecules in a thin film of vitrified buffer, which is introduced into an electron microscope. The instrument records images with an electron-sensitive detector, such as a photographic film or charge-coupled device camera. High-resolution 3D images can be recorded from 2D crystals, helical filaments, or icosahedral viruses, which have highly symmetric structure. For complexes without high symmetry or with a size smaller than 1000 kDa, however, resolution has not exceeded 5–20 Å. With the use of a digital camera, in which high speed is enabled by the ability to detect electrons directly, without first converting them to visible light, Bai et al. record a video of the sample during the time it is being exposed to the electron beam (typically ~1 s). This allows resolution-degrading movements of biomolecules induced by the beam to be compensated. They study ribosomes with this system and observe a resolution of ~4 Å. Furthermore, this is achieved with 100× fewer particles than the previous best cryo-EM resolution of ribosomes, 5.5 Å. Li et al. correct for beam-induced motions by using a new generation of complementary metal-oxide semiconductor cameras to detect electrons directly. They study 20 S proteasomes from *Thermoplasma acidophilum*. These particles have a molecular weight of 700 kDa and exhibit sevenfold symmetry (substantially smaller in size and lower in symmetry than icosahedral viruses). Li et al. achieve 3.3 Å resolution and are able to see side-chain densities clearly. Such studies open the possibility that cryo-EM will become broadly applicable to structural analysis of proteins at near-atomic resolution.

PROTEOMICS

Shliaha P V, Bond N J, Gatto L, Lilley K S. Effects of traveling wave ion mobility separation on data independent acquisition in proteomics studies. *Journal of Proteome Research* 12;2013:2323–2339.

The depth of proteome coverage in terms of peptide identification and peptide quantification during liquid chromotography (LC)-tandem mass spectrometry is limited by the efficiency with which peptides can be separated by LC and isolated on the basis of a mass:charge ratio in the gas phase for fragmentation. The introduction of commercial mass spectrometers, equipped with a traveling waveform ion mobility sector that separates precursor ions by their mobility in the gas phase, has ameliorated this problem without increasing instrument run times. Shliaha et al. document the effect of ion-mobility separation on proteome analysis in a Waters Synapt G2 instrument, in which the ion mobility sector is interposed between the quadrupole ion trap and time-of-flight (TOF) sector in a quadrupole TOF mass spectrometer. They show that use of ion-mobility separation increases the number of peptides confidently identified in data-independent acquisi-

tion mode, as it increases the ability to assign product ions to distinct precursors, thereby improving accuracy of identification. The improvement is noted specifically for peptides in the low-abundance range. Use of ion-mobility separation decreases sensitivity slightly, as ion transmission losses occur at the interface between the low-pressure quadrupole sector and the high-pressure ion-mobility sector. More importantly, however, with ion-mobility separation, there is a reduction in the linearity of signal-strength response at the upper end of the dynamic range. This is a result of detector saturation, resulting from the concentration of high-abundance ions by the quadrupole. Quantification of the peptides with highest signal intensity is thereby compromised. Where necessary, this problem could be addressed by decreasing the amount of sample analyzed, by performing separate runs with and without ion-mobility separation, or by analyzing distortion of signal intensities within isotopic envelopes.

Sköld K, Alm H, Scholz B. The impact of biosampling procedures on molecular data interpretation. *Molecular & Cellular Proteomics* 12;2013:1489–1501.

The concept of technical variation in experimental procedures is well-understood, and its assessment by replicate analyses is well-established. The role of biological sources of variation is less well-appreciated, especially in the context of samples procured within the research laboratory, as distinct from the clinic. For example, stress responses to changes in oxygen tension or temperature after sample preparation begins are able to cause large changes in sample composition in a short time. Also, continued activity of endogenous enzymes after cell disruption can affect post-translational modification very rapidly. Sköld et al. review such effects in considering what sample quality implies within the context of proteomic studies. The adequacy of sample preparation methods must be judged in light of the purpose of each study. For example, when biomarker discovery is the main goal, representativeness of disease state is the most important characteristic of samples, and a prolonged interval before inactivating endogenous enzymes might help reduce variation as a result of rapid ex vivo changes. In mechanistic studies, however, representativeness of the presampling state may be more important. Here, shortening the interval before inactivation of endogenous enzymes might paradoxically increase variability as a result of rapid ex vivo changes. The review of Sköld et al. will therefore be of interest to any investigator involved in interpreting molecular biology and biomarker data.

FUNCTIONAL GENOMICS AND PROTEOMICS

Laufer C, Fischer B, Billmann M, Huber W, Boutros M. Mapping genetic interactions in human cancer cells with RNAi and multiparametric phenotyping. *Nature Methods* 10;2013:427–431.

Roguev A, Talbot D, Negri G L, Shales M, Cagney G, Bandyopadhyay S, Panning B, Krogan N J. Quantitative genetic-interaction mapping in mammalian cells. *Nature Methods* 10;2013:432–437.

Kampmann M, Bassik M C, Weissman J S. Integrated platform for genome-wide screening and construction of high-density genetic interaction maps in mammalian cells. *Proceedings of the National Academy of Sciences USA* 110;2013:E2317–E2326.

Genetic interaction mapping seeks to garner information about how genes collaborate to produce phenotypic characteristics. A biological system, such as a yeast or bacterial cell, is perturbed by mutation in a gene, and the impact of the mutation on a measurable phenotype, such as cell size, is measured. The same phenotypic character is then measured following second-site mutations in other genes to determine the relationships between the genes in the biochemical pathways, protein complexes, etc., which they affect. Large-scale application of this methodology to mammalian cells is impeded by genomic complexity and lack of extensive libraries of mutants. Three groups have now developed methods for large-scale genetic interaction screening in mammalian cells based on combinatorial RNA interference (RNAi). Laufer et al. look for genetic interactions between chromatin-remodeling genes using endonuclease-prepared short interfering RNAs. Roguev et al., who also study chromatin-remodeling genes, use commercial RNAi reagents. Kampmann et al. use a pooled, multicomponent library of short hairpin RNAs (shRNAs) in a lentiviral vector and use deep sequencing to ascertain the frequencies of shRNA-encoding cassettes to identify shRNAs relevant to a particular perturbation and the genes they hit. Kampermann et al. report data on cellular resistance to ricin or Shiga toxin. A library, expressing all pairwise combinations of double shRNAs that they identify, is then constructed and used to screen for phenotypes. The three research groups develop measurement procedures and computational approaches to identify genetic interactions in large datasets, provide methods to control for off-target effects of RNAi, and measure efficiency of gene knock-down. It is hoped that the tools they describe will provide ways to dissect functional interactions in mammalian cells, similar to those that have proved so effective in prokaryotes and yeast.